

## Video Article

# Rapid Protocol for Preparation of Electrocompetent *Escherichia coli* and *Vibrio cholerae*

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URL: <http://www.jove.com/video/50684>

DOI: [doi:10.3791/50684](https://doi.org/10.3791/50684)

Keywords: Bioengineering, Issue 80, Cell Engineering, Gram-Negative Bacteria, Enterobacteriaceae, Escherichia, *Escherichia coli*, Vibrionaceae, *Vibrio*, *Vibrio cholerae*, Bacteria, *Escherichia coli*, *Vibrio cholerae*, electrocompetence, transformation protocol, electroporation

Date Published: 10/8/2013

Citation: Gonzales, M.F., Brooks, T., Pukatzki, S.U., Provenzano, D. Rapid Protocol for Preparation of Electrocompetent *Escherichia coli* and *Vibrio cholerae*. *J. Vis. Exp.* (80), e50684, doi:10.3791/50684 (2013).

## Abstract

Electroporation has become a widely used method for rapidly and efficiently introducing foreign DNA into a wide range of cells. Electroporation has become the method of choice for introducing DNA into prokaryotes that are not naturally competent. Electroporation is a rapid, efficient, and streamlined transformation method that, in addition to purified DNA and competent bacteria, requires commercially available gene pulse controller and cuvettes. In contrast to the pulsing step, preparation of electrocompetent cells is time consuming and labor intensive involving repeated rounds of centrifugation and washes in decreasing volumes of sterile, cold water, or non-ionic buffers of large volumes of cultures grown to mid-logarithmic phase of growth. Time and effort can be saved by purchasing electrocompetent cells from commercial sources, but the selection is limited to commonly employed *E. coli* laboratory strains. We are hereby disseminating a rapid and efficient method for preparing electrocompetent *E. coli*, which has been in use by bacteriology laboratories for some time, can be adapted to *V. cholerae* and other prokaryotes. While we cannot ascertain whom to credit for developing the original technique, we are hereby making it available to the scientific community.

## Video Link

The video component of this article can be found at <http://www.jove.com/video/50684>

## Introduction

Since its' inception in the early 1980s<sup>1</sup>, electroporation has become an integral molecular biology technique, likely the single most common method employed to transform/transfect live cells with circular or linear nucleic acids. Originally developed for transfection of eukaryotic cells<sup>1</sup>, electroporation was subsequently adapted for transformation of *E. coli*<sup>2,3</sup>. In bacteriology, electroporation has become the laboratory standard and has been modified to transform a wide range of bacteria including Gram-negative *Pseudomonas*<sup>4</sup>, *Salmonellae*<sup>5</sup>, *Vibrios*<sup>6</sup>, *Serratiae*, and *Shigellae*<sup>7</sup>; Gram-positive *Clostridia*<sup>8</sup>, *Bacilli*<sup>9</sup>, *Lactobacilli*<sup>10</sup> and *Enterococci*<sup>11</sup>. Even some Archaeabacteria have proven amenable to transformation by electroporation, including *Methanococci*<sup>12</sup> and *Sulfolobus* species<sup>13</sup>. For a comprehensive review please refer to Aune and Aachmann<sup>14</sup>. Efficiency of transformation by electroporation is reportedly 10-20 fold higher than chemical competence or heat shock<sup>2</sup>. However, much like preparing bacteria for chemical competence as optimized by Douglas Hanahan in the 1980s<sup>15</sup>, cells must also be prepared to be electrocompetent.

Electroporation is a rapid and effective means of bacterial transformation, requiring electrocompetent bacteria, purified DNA, a pulse control module that delivers the electrical impulse, and a chamber that accommodates small disposable cuvettes that function as an electrode. Briefly, cold, competent bacteria are mixed with plasmid DNA, subjected to a high-voltage pulse in a cuvette, resuspended in growth media, incubated at 30-37 °C for 30-45 min, and then plated on semisolid medium (nutrient agar plates) with the appropriate selective antibiotic.

In contrast to the pulsing step, the preparation of electrocompetent *E. coli* remains a multipart procedure that is traditionally carried out in large volumes. Briefly, an overnight culture of bacteria is inoculated into an Erlenmeyer flask with 100 ml to 1 L Luria Broth (LB), grown to mid-logarithmic phase of growth (OD<sub>600</sub> of <1.0), and subjected to serial washes with a sterile non-ionic buffer or double-distilled water (ddH<sub>2</sub>O) in decreasing volumes at 4 °C. Great care must be taken to keep the cells cold throughout the entire procedure and prevent contamination. This requires the use of autoclaved centrifugation buckets and non-ionic buffers or ddH<sub>2</sub>O, an orbital shaker incubator, a large volume high-speed refrigerated centrifuge and a set of rotors. Bacteria are finally resuspended in a small volume of buffer supplemented with 10% glycerol and aliquoted into ~40 µl volumes, which are stored at -80 °C until use. Low-temperature storage often results in decreased transformation efficiencies due to loss of viability over time.

Electrocompetent bacterial cells are also available from a variety of commercial sources but only for a limited number of (often recombination-deficient) *E. coli* strains commonly employed as hosts to propagate a wide range of plasmids. As a result researchers rely on in-house methods to prepare their own strains/mutants for transformation.

An alternate, rapid and efficient protocol for the preparation of electrocompetent *E. coli* has been in use by a few molecular bacteriology laboratories for some time now and has been extended to additional Gram-negative bacteria, in our case *V. cholerae*. The originator of the protocol cannot be identified as it has been optimized by other researchers over time. The method presented here has been used in our laboratories for nearly two decades, and it is our goal to share this useful protocol with our peers.

## Protocol

### 1. Preparation of Bacterial Cultures, Tools, and Reagents (DAY 1, Afternoon)

1. In the late afternoon, inoculate 1-5 ml autoclaved LB broth in sterile (for example, 100 x 13 mm) borosilicate glass test tubes with a small aliquot of bacteria (*E. coli* or *V. cholerae*).
2. Set the inoculated test tubes in a roller drum housed at 37 °C temperature (warm room or incubator), turn on the roller drum at high speed, and incubate O/N.
3. Prepare a cell spreader in the shape of a "hockey stick" from a glass rod by heating the middle of the rod in the flame of a Bunsen burner until the glass softens and then direct the bend gently with tweezers or pliers into a 135° angle. Heat the rod once more in the middle point between the end and the first bend and bend it at a 45° angle inwards (in the opposite direction of the first bend).
4. Prepare LB-agar without antibiotics and pour into Petri dishes in preparation of the electrocompetence protocol, and LB-agar plates containing the appropriate antibiotic (according to the resistance marker on the vector to be electroporated), and store at 4 °C.
5. Prepare a filter sterilized 2 mM CaCl<sub>2</sub> solution for *V. cholerae*, or autoclave ddH<sub>2</sub>O for *E. coli*, and store at 4 °C.

### 2. Growth of Electrocompetent Bacteria (DAY 2, Morning)

1. Deliver 100 µl of the O/N bacterial culture onto each LB agar plate, frozen glycerol stocks may also be utilized and directly delivered onto the plate.
2. Dip the hockey stick-shaped cell spreader in 100% EtOH, briefly drain and burn off the remaining EtOH to sterilize it prior to use and spread. Spread the 100 µl O/N bacterial culture evenly with the sterile glass cell spreader making sure not to disrupt (break) the agar surface.
3. Incubate the plate at 37 °C for 4-6 hr, or until a thin lawn of bacterial growth becomes distinguishable. Cells are most competent when actively growing.

### 3. Preparation of Electrocompetent Bacterial Cells (DAY 2, Afternoon)

1. Harvest the bacteria with a sterile inoculating loop making sure not to pierce or break the surface of the agar. One 2 mm diameter bacterial mass is sufficient for a single transformation. Typically, this requires scraping the surface of the thin lawn with the inoculating loop two or three times. Several samples (typically between 4-6) may be collected from the same plate.
2. Resuspend the bacterial mass in 1 ml ice-cold 2 mM CaCl<sub>2</sub> (for *V. cholerae*) or sterile ddH<sub>2</sub>O (for *E. coli*) and mix well until no clumps are visible, keep on ice.
3. Centrifuge each bacterial suspension for 5 min at 5,000 x g in a refrigerated microcentrifuge set to 4 °C, or in a microcentrifuge stored in a cold room set to 4 °C.
4. Discard the supernatant, resuspend the bacterial pellet in the same volume of ice-cold 2 mM CaCl<sub>2</sub> or sterile ddH<sub>2</sub>O, and repeat the centrifugation step as done before twice more for a total of three washes.
5. Remove supernatant, resuspend, and then loosen the bacterial pellet thoroughly in 40 µl ice cold 2 mM CaCl<sub>2</sub> or sterile ddH<sub>2</sub>O and keep on ice.

### 4. Transformation of Electrocompetent Bacteria (DAY 2, Afternoon)

1. Add up to 1 µg of plasmid DNA (in up to 1 µl water or Tris-EDTA buffer) to the 40 µl bacterial suspension and transfer this mixture into a pre-chilled, sterile 0.2 cm gap cuvette. The salt concentration in the DNA sample must be low<sup>a</sup>, as it will contribute to arcing of the pulse in the next step.
2. Insert the cuvette into the electroporation chamber of the pulse control module, and electroporate at 1.8 kV, 25 µF. The time constant should be ~5.0 msec, and no arcing should occur.
3. Quickly recover the cell suspension by resuspending into 1 ml LB broth and transfer into previously autoclaved borosilicate glass test tube.
4. Allow the cells to recover by incubating under aerated growth conditions (in a roller drum) at 37 °C for 30 min without antibiotic selection.
5. Plate the bacteria onto the previously prepared LB agar plates in the presence of the appropriate selective agent (antibiotic), and incubate at 37 °C O/N. If bacteria are transformed with a high concentration of purified vector (0.1-1 µg supercoiled plasmid), deliver 10 µl of bacterial culture onto the edge of the LB agar plate and with a sterile inoculating loop streak for isolated colonies using the quadrant streak method. If a ligation mixture was transformed, deliver 100 µl of bacterial culture onto each agar plate and evenly spread it with the sterilized glass cell spreader.

<sup>a</sup> If the time constant is short (below 3.5 msec), or pulsing leads to arcing, then re-precipitate the DNA sample, wash with 70% EtOH twice to remove salts before drying and resuspending in TE buffer.

## Representative Results

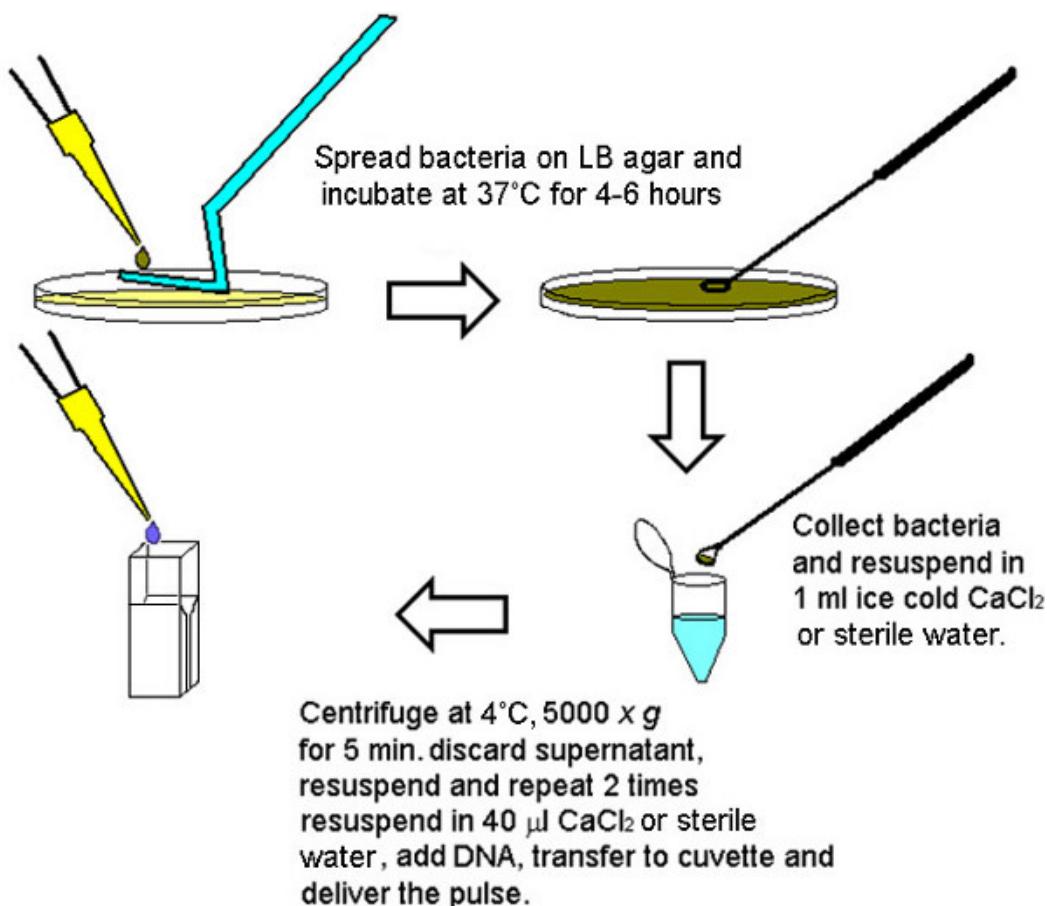
We carried out a set of transformations with *E. coli* and *V. cholerae* to compare transformation efficiency of our rapid methodology with an adaptation of the (traditional) method originally described by Dower *et al.*<sup>2</sup> for the preparation of electrocompetent bacteria. To carry out the traditional preparation of competent cells, 500 ml LB in a 2 L-Erlenmeyer flask were inoculated with 500  $\mu$ l overnight culture of *E. coli* DH5 $\alpha$  or *V. cholerae* O395, incubated in an orbital shaker (215 rpm at 37 °C) and harvested at OD<sub>600</sub> of between 0.5-1.0. The flask was cooled on ice and the cultures centrifuged in a pre-chilled rotor at 4,000 x g for 10 min at 4 °C. The cell pellets were thoroughly resuspended in 500 ml ice-cold 2 mM CaCl<sub>2</sub> for *V. cholerae* and ddH<sub>2</sub>O for *E. coli*, and centrifuged again under the same conditions. Subsequent rounds of resuspension and centrifugation were carried out in decreasing volumes of 200 and 100 ml volumes making sure the culture remained chilled at 4 °C. Finally, a resuspension and centrifugation in a 10 ml volume was carried out with 10% glycerol. The final pellet was resuspended in 2 ml 10% glycerol and 40  $\mu$ l aliquots were frozen at -80 °C and stored no longer than one week prior to electroporation.

Electroporation conditions including pulse settings and cuvette size were identical for both bacterial species in all electroporations regardless of method of preparation of competent cells. The only difference was that we carried out the washes in cold ddH<sub>2</sub>O for *E. coli* and cold 2 mM CaCl<sub>2</sub> for *V. cholerae*. **Table 1** shows results of bacteria transformed by electroporation identically but rendered competent either with the rapid protocol or with the traditional method. We employed strain O395 and DH5 $\alpha$  as commonly used, representative strains of *V. cholerae* and *E. coli* respectively; similar results can be obtained with other strains of the same species (albeit not every single strain has been tested) and likely adapted to other Gram-negative bacteria and probably beyond.

To insure that equal cell numbers were present in each batch pulsed, electrocompetent bacteria generated utilizing the rapid method were pooled upon collection, mixed, kept homogenously suspended, and aliquoted in 40  $\mu$ l volumes shortly prior to electroporation. Electrocompetent bacteria generated utilizing the traditional method were treated similarly prior to freezing in 40  $\mu$ l aliquots. Following delivery of the pulse, each batch of 40  $\mu$ l electroporated bacteria was suspended into 400  $\mu$ l LB and serially diluted 1:10. One hundred microliters of each dilution were plated on LB agar in the presence of 100  $\mu$ g/ml ampicillin and spread using a sterile glass cell spreader and incubated at 37 °C overnight. To quantify the total number of bacteria employed in each transformation, 40  $\mu$ l volumes of electrocompetent bacteria of each prepared batch were serially diluted, and plated on LB agar without antibiotics identically in parallel. One aliquot of equally treated bacteria from each batch was electroporated with 1  $\mu$ l Tris-EDTA (TE) buffer but without DNA (mock transformation), serially diluted and plated on LB agar without antibiotics to estimate the number of cells lost by the delivery of the electric pulse. Lastly, one additional variation to the experiment was carried out to determine whether 30 min incubations at 37 °C in 1 ml LB after electroporation but prior to plating on LB-agar would affect the recovery of transformed cells. colony forming units (CFUs) were enumerated on the following day.

For this experiment we employed pUC18, a common laboratory plasmid, and batches of competent bacteria consisting of approximately 10<sup>8</sup> CFUs for cells prepared utilizing the traditional method and 10<sup>7</sup> CFUs for cells prepared utilizing the rapid protocol. Electroporation alone (without DNA) reduced viable CFUs by 10-100 fold irrespective of the method used to prepare the electrocompetent cells. As shown in **Table 1**, transformant yield was within the 10<sup>6</sup>-10<sup>7</sup> CFUs/ $\mu$ g of DNA range in three replicate experiments (standard deviations in parentheses) for *E. coli* (DH5 $\alpha$ ) and *V. cholerae* (O395) respectively using the traditional, lengthier method for electrocompetent cell preparation. Transformant yield appeared decreased 10 fold to 10<sup>5</sup>-10<sup>6</sup> CFUs / $\mu$ g of DNA in three replicate experiments (standard deviation in parentheses) for *E. coli* and *V. cholerae* respectively using the rapid method. For this reason we determined the percentage of transformed bacteria by dividing transformed CFUs by the number of CFUs recovered from "mock transformations" (without plasmid) from otherwise identical batches of competent cells. The percentage of transformed bacteria was within one log (2.5-9.4%) regardless of method of preparation and species transformed (**Table 1** bracketed numbers). These results suggest that the efficiency of the rapid method is comparable to that of the traditional lengthier procedure of preparing competent cells and that the representative strains of *Vibrio cholerae* and *Escherichia coli* are equally amenable to the rapid procedure.

We found that transformation efficiency, for plasmids like pUC18 harboring  $\beta$ -lactamase cassettes, is not affected by the 30 min recovery time in LB broth. The same number of transformants was recovered whether the cells were plated to LB agar in the presence of ampicillin directly, after electroporation, or whether they were allowed a 30-45 min recovery time in LB broth at 37 °C prior to plating. Our findings that ampicillin-resistance does not require outgrowth under nonselective conditions suggests that  $\beta$ -lactamase expression occurs sufficiently fast upon transformation. However, it should be noted that we carried out serial dilutions of pulsed bacteria in LB broth, which may have contributed to recovery from the electric shock and initiate gene expression.



**Figure 1. Graphical depiction of the methodology for the rapid preparation of electrocompetent bacteria.** After 4-6 hr (depending on the density of the inocula) sufficient numbers of bacteria can be collected to carry out four to six transformations from a single LB agar plate. To insure equal number of competent cells for each final volume to be electroporated, bacteria collected from the LB agar plate were initially pooled and washed together then aliquoted in 40  $\mu\text{l}$  volumes depending on the size of the pellet (larger pellets were diluted in larger volumes divisible by 40  $\mu\text{l}$ ).

Number of Ampicillin-resistant transformants/ $\mu\text{g}$ DNA (Ampicillin-resistant transformants recovered [%])		
Strain	Traditional method	Rapid method
<i>E. coli</i> (DH5α)	$2.3 \times 10^6$ ( $\pm 7.3 \times 10^5$ ) [9.4%]	$3 \times 10^6$ ( $\pm 7.1 \times 10^5$ ) [2.5%]
<i>V. cholerae</i> (O395)	$4 \times 10^7$ ( $\pm 1.7 \times 10^7$ ) [5%]	$6.7 \times 10^5$ ( $\pm 2.1 \times 10^5$ ) [3.3%]

**Table 1. Transformation efficiency per microgram of DNA (pUC18) of electrocompetent bacteria prepared by the traditional method compared to the rapid method.**

All transformations were carried out with 500 ng pUC18 DNA (~80% supercoiled DNA as visualized by 1% agarose gel electrophoresis) and the electroporated cells were serially diluted 10-fold in LB broth prior to plating for CFU on LB-agar with antibiotics. Controls of non-electroporated bacteria and of bacteria electroporated without plasmid, serially diluted and plated on LB agar without antibiotics were carried out for each set of transformations to determine the total number of viable bacteria prior and after pulsing. The electroporated controls represented the base line for the percent of viable bacteria successfully transformed.

## Discussion

Transformation efficiency is subject to a variety of factors regardless of the method employed to prepare competent cells. Analogous variables apply to this method; great care must be taken that once the cells are harvested from the agar plate such that they are maintained at constant cold temperature (no higher than 4 °C). The bacterial lawn must be actively growing at the time of collection; cells must be in mid-logarithmic phase of growth. Quality of DNA (i.e. contamination of salts) influences transformation efficiency. Specifically, when employing this method, the agar should not be broken as agar fragments in the cuvette will cause arcing of the pulse.

Most problems encountered with this technique are associated with two issues; collecting the bacteria during the proper window of time from the agar plate when they are actively growing. Collecting them too soon will yield insufficient cell numbers and will render the scraping off the LB agar plate frustrating because the "pellicle" they form is hard to lift off the surface of the plate. Collecting bacteria too late will decrease their

competence dramatically. The window of time will naturally vary depending on the density of the inoculum plated on the LB agar plates. The second critical step is to keep the bacteria cold (4 °C) from the moment they are lifted off the agar plate and resuspended in CaCl<sub>2</sub> or ddH<sub>2</sub>O until they are plated on the LB agar following transformation. The microfuge tubes containing the bacteria, cuvettes and buffer should be pre-chilled and kept on ice at all times until the bacteria are plated. However, additional problems can arise when the sterile ddH<sub>2</sub>O or CaCl<sub>2</sub> contain precipitate or are contaminated. For this reason we recommend to prepare this reagent fresh.

Troubleshooting transformation is facilitated by including controls when transforming bacteria rendered competent by any method. A negative control consisting of a batch of non-transformed competent bacteria plated on an LB agar plate containing the selective marker will rapidly help determine whether the antibiotic on the plate is effective. A positive control transformation with a known plasmid preparation of good quality and quantity harboring the same selective marker as the experimental sample will be helpful in determining whether the bacteria are competent but also whether the experimental DNA transformed was sufficient in quantity or quality.

The advantages of this technique are multiple; the method can be carried out within the same day that a transformation is planned for. We have found that frozen bacterial glycerol stock may be plated directly on the LB agar without any loss in electrocompetence; we have done this in "emergency" situations (after forgetting to start the overnight culture the day before). Although we could not test every strain known, any *E. coli* or *V. cholerae* strain that can be transformed by electroporation has been amenable to this protocol in our hands. The technique is rapid, effective, and yields transformation efficiencies comparable to those obtained from preparing electrocompetent bacteria employing traditional methods. The method eliminates the requirement for high speed centrifuge and associated rotors, an orbital incubator shaker and sterile centrifuge buckets (containers), and large volumes of autoclaved buffer. Although the Table of Specific Reagents and Equipment is quite comprehensive, most laboratories that carry out electroporation will have most, if not all, of these materials already available. Perhaps most importantly, the technique can be readily applied to generate electrocompetent laboratory-specific strains with mutant backgrounds rapidly. We are unaware of any limitations of this technique as it has replaced the traditional method of preparation of electrocompetent bacteria in our laboratories.

The results of our experiments shown here were carried out with fresh electrocompetent cells produced with the traditional method that had been stored at -80 °C for no longer than 1 week. However, frozen electrocompetent cell stocks lose competence and or viability over extended periods of storage. Our abridged protocol permits the preparation of fresh electrocompetent cells the same day the transformation is planned without concerns about age of the stock which may result in decreased transformant yield. We have not attempted storing electrocompetent cells produced using the rapid method for use on another day as this has not been necessary.

While not shown here, the efficiency of the electrocompetent cells prepared using our rapid method is sufficient for ligation mixtures. The transformations reported for non-O1 *V. cholerae* in a recent article by Unterweger *et al.*<sup>17</sup> were carried out using electrocompetent bacteria prepared by the method described here (except those carried out by conjugation). This technique affords researchers to transform strains with mutant backgrounds rapidly and effectively without having to prepare large batches of cells. This rapid preparation of electrocompetent bacteria method might be adapted to other prokaryotes that have been shown to be amenable to electroporation as the principle of preparation and the electroporation settings are going to be the same as those of traditional protocols.

## Disclosures

No disclosures are applicable.

## Acknowledgements

The authors are grateful to their colleagues and staff of present and past laboratories that have supported the development of this technique. S.P.'s laboratory is supported by the Canadian Institute for Health Research Operating Grant MOP-84473 and Alberta Innovates-Health Solutions (funded by the Alberta Heritage Foundation for Medical Research Endowment Fund). D.P.'s was supported by National Institutes of Health grants MD001091-01 and GM068855-02.

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